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DEPARTMENT OF THE ARMY  
Fort Detrick  
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Immunology - Method of immunochemical analysis by specific precipitation in  
a gelled medium, by Jacques M. Oudin

When one places in a tube a layer high enough of a solution rather concentrated of an antigen above a layer of homologous rabbit immune serum (<sup>1</sup> We take for instance in this study the immune serum of the rabbit because its precipitation is not inhibited by an excess of antibodies, as is the case with horse antiproteids) previously gelled by means of gelatin or better agar, and, if one keeps this tube at a constant temperature, there appears in the layer of immune serum an opaque zone progressively more noticeable toward the top (inhibition by excess of antigens) (<sup>2</sup> We present here only the first of three techniques that we have considered, the two others being: a, gelled antigen surmounted by a layer of immune serum; b, antigen and immune serum placed on either side of a column of gel); IN THE CASE of the ovalbumine and probably in the case of the mono dispersed antigens or haptens, this area is limited toward the bottom in a clear manner; in the case of the polyside or pneumococcus (type VIII), and probably in the case of the antigens or poly-dispersed haptens, the lower limit progressively diminishes.

This opaque area, whose opacity is a function of the richness of the layer with fewer antibodies, ~~shifts~~ shifts, and the relation of this shift  $h$  (easily measured when the lower limit is clear) with the square root of the time  $\sqrt{t}$  appears constant in a quite satisfactory manner, in accordance with the law of Fick. The relation  $h/\sqrt{t}$  increases, if without changing the other conditions of the experiment, the concentration of the antigen is increased or if the antibodies are diluted (<sup>3</sup> There is a need to pin down the laws of these phenomena. The method will apply perhaps (alors?) to quantitative data concerning the diffusion of the antigens studied.) These phenomena will permit, by comparison with the standards, a quantitative evaluation either of the antibodies or of the antigen of a precipitant system.

If one studies in one same tube the two separate precipitant systems as examples, by mixing ovalbumine and "polyside" in the higher layer, and the two immune serums <sup>respective homologous</sup> in the lower gelled layer, the two areas opaque areas each form by themselves and the tube appears as to these layers, as the superposition of two separate proofs.

Further, when one uses, as an antigen in the higher layer, a biologically antigenic liquid and, in the lower gelled layer, the antiserum obtained by immunizing an

the same biological liquid, if one observes in the lower layer many opaque areas, one could conclude that the liquid being studied contained at least at many different antigenic substances as there were different opaque areas not found in the controls. While studying in this manner the reaction of a normal horse serum with a mixture of serums of rabbits immunized for a long time intravenously against the horse serum, we have observed, in the lower layer of gelled antiserum, five opaque areas or, to state it better, five clearly differentiated regions. A picture of them could be placed on a sensitive plate if one used in place of a tube, a well (tank) with parallel sides. We will call these areas for the time being from the faster to the slower: A, B, C, D, E; a careful examination at different times of the tubes in which ~~was~~ the concentration of the antiserum was varied, would cause one to say that many of these opaque areas are not distinct units in themselves ("simple"), but result from the superposition or juxtaposition of more distinct (ie, self-contained) areas. These five opaque areas are subdivided thus: A, B<sub>1</sub>, B<sub>2</sub>, C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, D, E. The horse serum under study contains then at least eight distinct antigenic substances (of which five or less are of more or less different specifications). These figures are minimums, not so much because of the possibility that two opaque areas might be hidden by each other, but because the very rare substances in the serum studied or the very weak antigens are not manifested; they probably would increase by use of certain modification of the technique of by the use of immune serums provided by other animals.

This method of analysis permits further the identification of the antigen responsible for a given zone by means of an antigen contained in a more or less pure state in a portion of the same biological liquid: 1) if one draws out the immune serum by an antigen contained in the portion, the opaque area caused by this antigen <sup>would</sup> not appear; 2) if one should enrich the biological liquid by adding to it the portion in sufficient quantity, each opaque area whose swiftness of progression is increased in relation to the control corresponds to an antigen contained in this portion. The harmonious results of the two techniques have shown us for example that, in the ~~system~~ multiple precipitant system given above, area A corresponds to an antigen contained in crystallised serum albumin.

The results of the enumerations obtained by this method, working only with very sensitive biological reactants, should be compared with those of already existing methods, such as widening ("relargage"), ultracentrifugation, ultrafiltration, electrophoresis, which furnish, by totally different means, an enumeration (differentiation) of the large molecule substances contained in a mixture.

At ...

L.B.